PIER Energy-Related Environmental Research
Environmental Impacts of Energy Generation, Distribution and Use

Molecular Identification and Enumeration of Invertebrate Larvae Potentially Entrained by Once-Through Cooling in Morro Bay and Elkhorn Slough, California

Contract #: 500-04-025
Contractor: Moss Landing Marine Laboratories
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Matching Funds: $14,100
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The Issue

Approximately 45 percent of California's generation capacity is provided by facilities located along the state's coast and estuaries using once-through cooling technology. This cooling technology requires the withdrawal of significant amounts of water (~17 billion gallons per day) that is passed by the condenser and then discharged back into a waterbody. Although some of these facilities have been operating since the 1950s, scientific understanding of the ecological effects of the use of once-through cooling is quite limited. The impacts of cooling water withdrawals are characterized as *entrainment,* where small aquatic organisms are carried by the cooling water into the power plant and killed by heat, and as *impingement,* where the cooling water intake traps larger organisms against the intake screens.

New Clean Water Act 316(b) regulations require entrainment and impingement sampling. Of the species sampled, only a few can be identified by morphological characteristics. Predicting community impacts requires species-level identifications. However, traditional methods of planktonic analysis are inadequate to this task because species-level identification guides are few and incomplete. Models that predict impacts of cooling losses on estuarine communities depend on input of reliable population data. Thus there is a critical need for development of efficient and reliable methods of identification and enumeration of plankton.

Project Description

Moss Landing Marine Labs will conduct work using genetic markers to identify invertebrate larvae. Genetic markers for identification are based upon analysis of planktonic DNA (Figure 1), and have attractive qualities. First, genetic markers do not change during growth of the organism through its life stages, so they can be applied in any stage of development. Second, molecular methods for detecting genetic markers can be automated, which could lead to high-throughput protocols for plankton analysis. Research on methodology is required before molecular methods can be routinely used in plankton analysis. The purpose of this research is to expand current

knowledge and technology regarding genetic marker identification in order to develop more efficient identification techniques that will be useful in studies of entrainment and impingement.

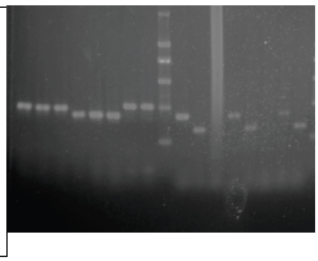

Figure 1. Example of species-specific cytochrome oxidase b amplification from bivalve larvae from multiplex PCR. Each lane represents a PCR reaction containing multiple primer pairs but DNA only from *Protothaca staminea* (lanes 1-6) or *Tresus nuttali* (lanes 10-17). (Different sized bands for each species result from different tested primer sets). Each single band indicates that amplification occurred with only the primers matching the added template. Only one PCR product is produced, indicating that primer pairs do not amplify nonspecifically. Lanes 7 -8 are positive controls with universal primers, and lane 9 is a size marker. Lane 12 is overamplified and unscorable; lane 15 is a failed amplification and unscorable.

Research for this investigation will encompass the following goals:

1. Develop a database of sequences for plankton in the source waters of Elkhorn Slough Estuary. A database of DNA sequences will be developed for the abundant planktonic organisms collected within the slough, focusing on bivalves, gastropods, decapod crustaceans, annelids, and other common taxa. Dominant adult bivalves, gastropods, annelids, and decapods within Elkhorn Slough will also be surveyed, to match larval sequences to adult sequences and ensure that DNS sequences are developed for all of the species.

2. Use Real Time-polymerase chain reaction (RT-PCR) for species identification of bivalve larvae in Morro Bay. The research will build upon previous investigations and will test the utility of two commercially available RT-PCR systems for efficacy and cost efficiency.

3. Determine the DNA content of individual larvae at different sizes or ages. This analysis will help identify and reduce data variability related to RT-PCR processing techniques. Factors considered will include nuclear gene copy number, as well as organism size, age, and physiological condition. The samples will be compared to known samples to determine DNA content.

4. Evaluate variation in RT-PCR due to variation in background DNA. Using known sample sizes the researchers will extract and analyze DNA in a dilution series in RT-PCR. This analysis will test the sensitivity of RT-PCR to DNA variation and differing levels of contamination associated with extraction methods.

The results of the research will be used to develop a database of DNA sequences for common California estuarine species, complete the sequencing to determine which species are the most abundant in the cooling water source waters of Morro Bay, provide information to modelers for input into impact-prediction models and evaluation the performance of DNA extraction methods. These goals will allow for identification of species that could otherwise not be identified by traditional methods using morphological characteristics through a microscope.

PIER Program Objectives and Anticipated Benefits for California

This project offers numerous benefits and meets the following PIER program objectives:

- **Improving the environmental costs/risk of California's electricity improving assessment.** By studying genetic markers of invertebrate larvae, power plant operators and regulators will be better able to identify and quantify their entrainment and impingement in once-through cooling systems. As a result, they will be better able to address environmental goals.

This work also conducts research and development activities that will advance science or technology not adequately addressed by the competitive and regulated markets that evaluate and resolve environmental effects of energy delivery in California.

Final Report

PIER-EA staff intend to post all the final project reports on the Energy Commission website as the research is completed (fall 2008 for the program final report and will list the website links here. All reports are also posted at the Water Intake Structure Environmental Research website http://ecomorphology.mlml.calstate.edu/WISER/.

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